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Effects of selective serotonin$_2$ ligands on behaviors evoked by stress in the rat

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ABSTRACT

Serotonin (5-HT) has been implicated in the regulation of the stress response. Two experiments were conducted to investigate the possibility that the 5-HT$_{2A,\ 2C}$ agonist DOI would reduce behavioral responsiveness to stress, and that selective blockade of one or both of these receptor subtypes would reverse this effect. Stressors employed were mild tail pinch and an illuminated open field. In Experiment 1 DOI (0.1, 0.5, 1.0 mg/kg, s.c.) was found to decrease stress-evoked oral behavior directed at food and to increase rearing behavior in a dose-dependent fashion. Neither of these effects was reversed by spiperone (5-HT$_{2A}$ antagonist) or SDZ SER-082 (5-HT$_{2C}$ antagonist). DOI also increased the frequency of head shaking. This effect was reversed by SDZ SER-082. In Experiment 2 DOI was injected singly or in combination with ketanserin (5-HT$_{2A,\ 2C}$ antagonist). DOI decreased tail pinch-evoked oral behavior directed at food, the amount of food eaten, and increased vocalization. In the open field DOI decreased rearing, increased the number of head shakes, and increased the incidence of flat body posture. While ketanserin alone (0.5, 2.5, 5.0 mg/kg) had no effect on any behavioral measure, coadministration of ketanserin (5.0 mg/kg) with DOI (0.5 and 1.0 mg/kg) significantly blocked the effects of DOI on oral behavior directed at food, eating, rearing, head shaking, and flat body posture. It is concluded that the observed effects of DOI on behaviors evoked by stress were mediated by activation of both 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors.

1. Introduction

Serotonin (5-HT) has been implicated in the etiology of anxiety and depression in humans and serotonin agonists have proven to be of benefit in the treatment of these disorders. Animal models of human anxiety have investigated behavioral responses to stressors in an attempt to determine which of the multiple receptor subtypes for serotonin might be of particular importance. The present studies focus upon the 5-HT$_2$ receptor. Three subtypes of the 5-HT$_2$ receptor are recognized. These are designated 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$. In addition to mediating peripheral actions of 5-HT such as bronchoconstriction, platelet aggregation, and muscle contraction (Hoyer et al., 1994), 5-HT$_2$ receptors have also been implicated in the regulation of the stress response. Whether activation of these receptors results in an increase or a decrease in reactivity to stress is a matter of current debate. Several studies have reported that systemic administration of selective 5-HT$_2$ *antagonists* reduces an animal's responsiveness to stress (e.g., Griebel et al., 1997; Kennett et al., 1997; Rademacher et al., 2002; Schreiber et al., 1998), while others have observed that 5-HT$_2$ *agonists* decrease behavioral responses to stress (e.g., Duxon et al., 1997; Massé et al., 2007; Nic Dhonnchadha et al., 2003a,b; Njunge and Handley, 1991; Sokal et al., 2005).

One commonly employed technique for investigating the behavioral effects of stressors in animals is the application of mild tail pinch. From its original description in 1975 (Antelman and Szechtman, 1975) tail pinch stress has been shown to reliably alter a variety of behaviors. Relative to non-stressed controls tail pinch has been reported to affect behaviors such as freezing (Giorgi et al., 2003), vocalization (Wax et al., 1975), grooming (Giorgi et al., 2003), and a number of oral behaviors directed at food, such as eating (Antelman and Szechtman, 1975; Czech et al., 1998; Fray et al., 1982; Samarghandian et al., 2003), gnawing (Fray et al., 1982; Giorgi et al., 2003), and licking (Czech et al., 1998). We have reported previously that DOI, a nonselective agonist of the 5-HT$_2$ receptor, reduces tail pinch-induced stress responding when injected subcutaneously, or after combined subcutaneous and intracerebroventricular administration (Hawkins et al., 2002).

The purposes of the present studies were to replicate our previous observation of reduced responding to stressors following systemic administration of DOI, and to explore which of the 5-HT$_2$ receptor subtypes may be mediating this effect. Two experiments were conducted. In Experiment 1 a series of four studies was completed. These examined stress responding following administration of DOI, a 5-HT$_{2A}$ antagonist (spiperone), a 5-HT$_{2C}$ antagonist (SDZ SER-082), and DOI when coadministered with these antagonists. Experiment 2 investigated the effect of a combined 5-HT$_{2A,\ 2C}$ antagonist (ketanserin)

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on stress responding, and the effect of this antagonist on the action of DOI. The affinities of these ligands for 5-HT_2 receptor subtypes are displayed in Table 1. Substantial affinities (K_i<115 nM) for other receptors are also provided.

Methods common to both experiments follow. Procedures unique to each experiment are described in their respective sections below. All protocols used in these experiments were reviewed and approved by the Louisiana State University Institutional Animal Care and Use Committee.

2. Methods

2.1. Animals

Male Sprague-Dawley rats (8–12 weeks) were obtained from the Division of Laboratory Animal Medicine at Louisiana State University and were housed individually with water and laboratory chow available ad libitum. The vivarium was maintained at 22 °C with lighting cycled on a 12:12 photoperiod (lights on at 07:00 h). Experimentation was conducted during the light phase.

2.2. Drugs

DOI ((+/−)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride) and ketanserin (KET) (3-(2-[4-[(Fluorobenzoyl)-1-piperidinyl] ethyl)-2,4(1*H*,3*H*)-quinazolinedione tartrate) were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Spiperone (8-[3-(*p*-fluorobenzoyl) propyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one) and SDZ SER-082 ((+)-cis-4,5,7a,8,9,10,11,11a-Octahydro-7*H*-10-methylindolo[1,7-bc][2,6]-naphthyridine), were obtained from Tocris Cookson Inc. (Ellisville, MO). Drugs were stored according to manufacturers' recommendations until the day of injection and were dissolved in sterile 0.9% saline just prior to injection. Saline was used for all control injections.

2.3. Injection procedure

An animal was weighed just prior to s.c. injection and the injection was administered at the base of the neck in a volume of 1.0 ml/kg. The animal was then returned to its home cage for 30 min prior to the initiation of stress testing. The 30 min delay was selected based upon previous research in our laboratory (e.g., Hawkins et al., 2002) which has shown DOI to exert a significant effect on stress responding at 30 min post injection. Existing literature has also shown systemic ketanserin to be effective within this time frame to alter a variety of dependent variables, including suppression of dopamine-induced hyperlocomotion and cocaine-induced 5-HT release (Boderick et al., 2004), reversal of DOI-induced head twitch (Miyata et al., 2004), and attenuation of DOI-induced peripheral vasoconstriction and hyperthermia (Blessing and Seaman 2003). Within 30 min after injection spiperone has been reported to increase ptosis, evoke ataxia, and suppress spontaneous tail-flick evoked by 8-OH-DPAT (Millan et al., 1994); block hyperlocomotion evoked by ethanol (Lê et al., 1997); and reverse apomorphine-induced reduction in startle responding (Swerdlow et al., 1991). SDZ SER-082 also acts within 30 min to augment cocaine-induced hyperlocomotion (Filip et al., 2004), decrease passive avoidance (Mora et al., 1997), and reduce body shakes produced by DOI (Dave et al., 2002).

Table 1
Receptor affinities, expressed as K_i (nM), for ligands used in the experiments

Receptor subtype	Ligand			
	DOI	Ketanserin	SDZ SER-082	Spiperone
Affinity for 5-HT_2 receptors				
5-HT_{2A}	2.51[a]	1.6[b]	512.86[c]	0.39[a]
5-HT_{2B}	27.54[a]	3,162.27[d]	204.17[c]	3,278.0[e]
5-HT_{2C}	3.01[a]	20.89[a]	7.58[c]	2,137.96[a]
Affinity (K_i<115 nM) for other receptors				
5-HT_{1A}	–	–	–	114.81[f]
5-HT_7	–	–	–	19.95[a]
α_1-adrenergic	–	15.00[g]	–	39.81[h]
Dopamine D_2	–	–	–	0.37[i]
Dopamine D_3	–	–	–	0.32[i]
Dopamine D_4	–	–	–	0.39[j]
Histamine H_1	–	1.79[k]	–	–

[a] Boess and Martin (1994).
[b] Toll et al., (1998).
[c] Knight et al., (2004).
[d] Glusa and Pertz (2000).
[e] Kursar et al., (1994).
[f] Millan et al., (1994).
[g] Bogeso et al., (1988).
[h] Schwinn et al., (1995).
[i] Tang et al., (1994).
[j] Roth et al., (1995).
[k] Ghoneim et al., (2006).

2.4. Tail pinch

Thirty minutes after s.c. injection, an animal was placed in the center of a suspended wire cage containing a pre-measured amount of laboratory chow and the animal's tail was guided through the cage floor. A clamp fashioned from hemostatic forceps, padded with latex tubing, was attached to the tail at a location which had been marked previously to correspond to a tail diameter of 4.3 mm. The animal's behavior was recorded for 4 min, after which the forceps were removed and the animal was returned to its home cage.

Changes in 7 behavioral variables were assessed:

1. *Oral behavior directed at food* — amount of time an animal licked, gnawed, or chewed laboratory chow
2. *Oral behavior not directed at food* — amount of time an animal licked or bit the cage or engaged in teeth chattering
3. *Grooming* — amount of time an animal preened its fur or tail with its mouth or forepaws
4. *Number of vocalizations*
5. *Defecation* — number of fecal boli
6. *Gnawing* — weight of shredded lab chow collected beneath the cage
7. *Eating* — difference in weight of lab chow prior to testing and weight of remaining intact and shredded chow after testing

Measurements of time for the above were to the nearest 0.01 s and measurements of weight were to the nearest 0.01 g.

2.5. Open field

At the conclusion of tail pinch testing each animal was returned to its home cage for 10 min prior to evaluation in an open field (61×61×61 cm) with a floor which was marked into 30.5 cm quadrants. An animal was placed into the field and observations of the following variables were conducted for 4 min:

1. *Line crosses* — number of lines crossed with all four paws
2. *Rearing* — number of times an animal lifted both front paws simultaneously from the floor
3. *Head shakes* — number of times an animal shook its head from side to side
4. *Wet dog shakes* — number of instances when an animal shook its head and upper torso
5. *Freezing* — amount of time an animal remained motionless
6. *Flat body posture* — occurrence (presence or absence) of an elongated posture in combination with a creeping gait. This variable was assessed in Experiment 2 only.

2.6. Rotarod

The rotarod consisted of a textured stainless steel drum (7.2 cm dia) which was set to rotate at 10 rpm. Twenty-four hours after the

conclusion of tail pinch and open field testing, each animal was trained on the apparatus until it could remain on the rotating drum for 30 s. Animals which did not reach this criterion within 12 successive trials were excluded from subsequent testing.

Rotarod testing occurred 24 h after training. Animals meeting the training criterion were administered drug or saline based on the experimental condition assigned to them on the first day of stress testing. Injections were administered as previously described. Animals remained on the rotarod for 30 s or until loss of balance, whichever occurred first. Latency to loss of balance, if it occurred, was recorded to the nearest 0.1 s.

2.7. Statistical analyses

Tail pinch and open field data were analyzed with multivariate analysis of variance (MANOVA). Significant results were followed by univariate analysis of variance (ANOVA) and the Least Significant Differences Test. Because some animals did not meet the training criterion, data collected on the rotarod were analyzed separately by univariate analysis of variance. As a nominal variable (presence or absence), flat body posture was analyzed by X^2 analysis. Alpha was set at 0.05 for all analyses.

3. Experiment 1

Experiment 1 was designed to evaluate the effects of a systemically administered 5-HT_2 agonist (DOI) on behaviors evoked by stress, and to determine whether such effects are differentially attributable to the 5-HT_{2A} or 5-HT_{2C} receptor subtype. A within-subjects protocol was used in which four groups of animals were subjected to 1) DOI alone (n=12), 2) a 5-HT_{2A} antagonist (spiperone) alone (n=10), 3) a 5-HT_{2C} antagonist (SDZ SER-082) alone (n=10), or 4) DOI in combination with one of these antagonists (n=8).

The three groups which received injections of a single drug were administered doses of 0.1, 0.5, and 1.0 mg/kg of the respective drug as well as a saline control injection. These drug doses are referred to as "low", "medium", and "high" doses in the presentation of results below. The group which received DOI in conjunction with an antagonist was exposed to the following four conditions: DOI alone (0.5 mg/kg), DOI (0.5 mg/kg) plus SDZ SER-082 (1.0 mg/kg), DOI (0.5 mg/kg) plus spiperone (1.0 mg/kg), and saline control. When animals received DOI in conjunction with either SDZ SER-082 or spiperone the agonist and antagonist were administered in a single injection in which the total volume was maintained at 1 ml/kg. For all groups the order of presentation of injections was manipulated via a modified Latin-square design and 3 days elapsed between subsequent injections.

An exclusionary criterion of 2 s of oral behavior directed at food during tail pinch in the saline condition was employed. Data from animals which did not meet this criterion were not subjected to statistical analysis. The number of animals per group reported above represents the final n/group after exclusion of non-responders.

4. Results

DOI alone. MANOVA revealed a significant effect of drug dose across the four treatment conditions [$F(39,96)=2.38$, $p<0.001$]. Post hoc analyses revealed that DOI significantly altered three behavioral variables: oral behavior directed at food, rearing, and head shaking. The duration of oral behavior directed at food during tail pinch stress was decreased in a dose-dependent fashion [$F(3,11)=7.12$, $p<0.001$]. These data are displayed in Fig. 1 (panel A). Although the low dose of DOI did not alter oral behavior, the medium ($p=0.003$) and high ($p<0.001$) doses significantly suppressed responding. The effects of the medium and high doses also differed significantly from the low dose ($p=0.012$ and $p=0.002$ respectively). An identical dose-related pattern was observed in regard to rearing in the open field [$F(3,11)=10.60$, $p<0.001$] (please see Fig. 2, panel A). Rearing was significantly reduced by the medium ($p=0.001$) and high ($p=0.001$) doses only, and the effects of these two doses were significantly different from the low dose ($p<0.001$ for both comparisons). Number of head shakes in the open field (Fig. 3, panel A) was significantly increased by DOI [$F(3,11)=5.21$, $p=0.002$)]. This effect was attributable to the medium dose only ($p<0.001$).

Spiperone or SDZ SER-082 alone. MANOVA revealed that neither spiperone [$F(33,77)=1.17$, $p=0.284$] nor SDZ SER-082 [$F(33,77)=1.01$, $p=0.466$] significantly altered behavioral responding when administered singly.

DOI coadministered with spiperone or SDZ SER-082. Drug administration significantly altered behavioral responding [$F(36,86)=1.82$, $p=0.012$]. Univariate analyses revealed effects on oral behavior

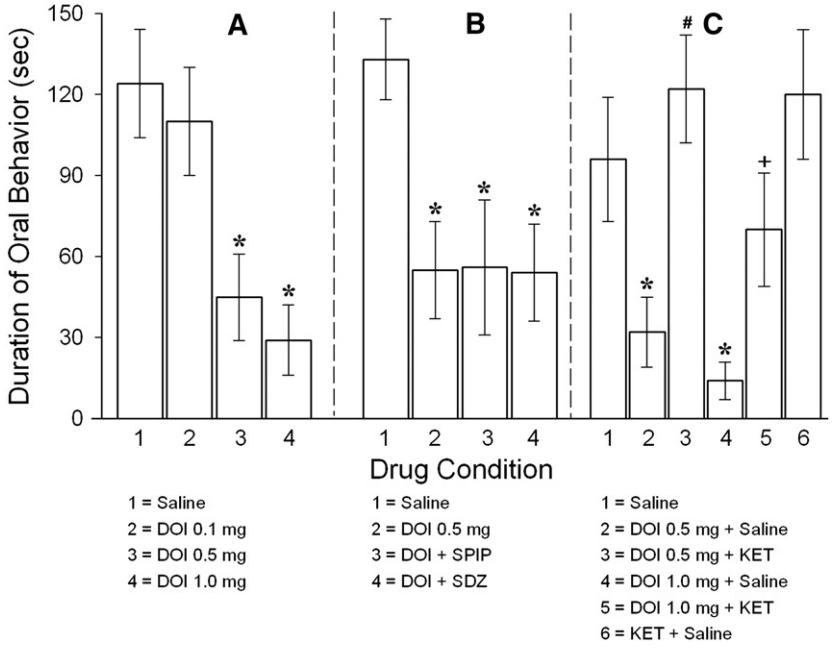

Fig. 1. Seconds of oral behavior directed at food during tail pinch stress following injections of saline control, DOI, spiperone (SPIP), SDZ SER-082 (SDZ), ketanserin (KET), or combinations of these. Panels A and B present data from Experiment 1 and panel C contains data from Experiment 2. Significance values: * = $p \leq 0.05$ relative to saline control, # = $p \leq 0.05$ relative to DOI 0.5 mg/kg + saline, + = $p \leq 0.05$ relative to DOI 1.0 mg/kg + saline.

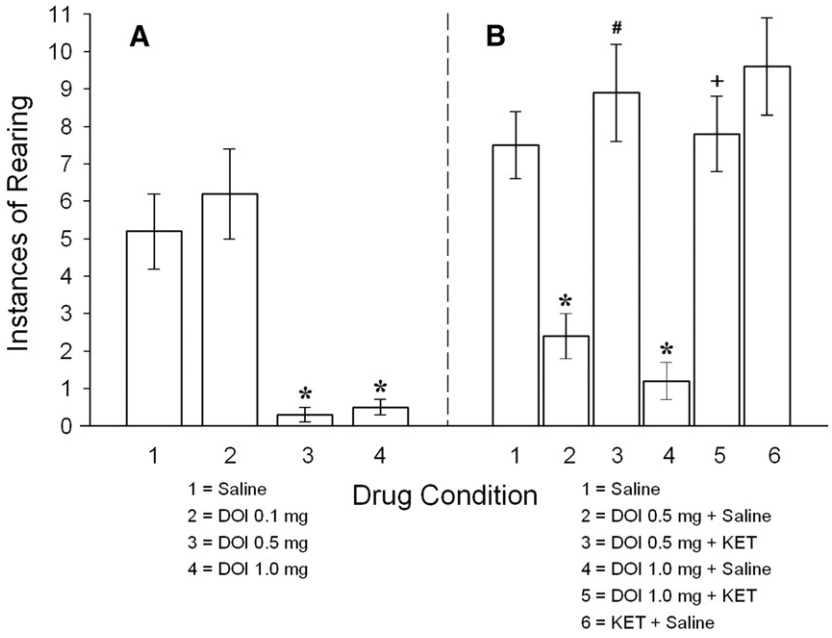

Fig. 2. Number of instances of rearing in the open field following injections of saline control, DOI, ketanserin (KET), or combinations of these. Panel A presents data from Experiment 1 and panel B contains data from Experiment 2. Significance values: * = $p \leq 0.05$ relative to saline control, # = $p \leq 0.05$ relative to DOI 0.5 mg/kg + saline, + = $p \leq 0.05$ relative to DOI 1.0 mg/kg + saline.

directed at food [$F(3,7)=4.00$, $p=0.01$], number of lines crossed [$F(3,7)=3.26$, $p=0.03$], number of head shakes [$F(3,7)=4.44$, $p<0.01$], and duration of freezing behavior [$F(3,7)=5.07$, $p<0.01$].

As was seen previously, oral behavior directed at food during tail pinch was significantly reduced by DOI ($p=0.007$). These data are displayed in Fig. 1, panel B. This effect of DOI was not reversed by coadministration of either spiperone ($p=0.008$) or SDZ SER-082 ($p=0.007$).

DOI increased the number of head shakes ($p=0.004$; see Fig. 3, panel B). This effect was not altered by concomitant injection of spiperone ($p>0.05$ relative to DOI alone, $p<0.001$ relative to saline) but was abolished by coinjection with SDZ SER-082 ($p=0.004$ relative to DOI alone, $p>0.05$ relative to saline).

The number of lines crossed in the open field was reduced by 50% when DOI was coinjected with SDZ SER-082 ($p=0.012$; saline mean = 14, drug mean = 7). Neither DOI by itself nor DOI plus spiperone had an effect on this variable (data not displayed).

Injections of DOI plus SDZ SER-082 increased the duration of freezing behavior from an average of 20 s following saline to 80 s. This effect differed significantly from the saline ($p=0.006$), DOI ($p=0.001$), and DOI plus spiperone ($p=0.004$) conditions. As with line crossings, neither DOI injected alone nor DOI coinjected with spiperone altered the duration of freezing behavior (data not displayed).

Rotarod. In no instance was rotarod performance affected by drug administration.

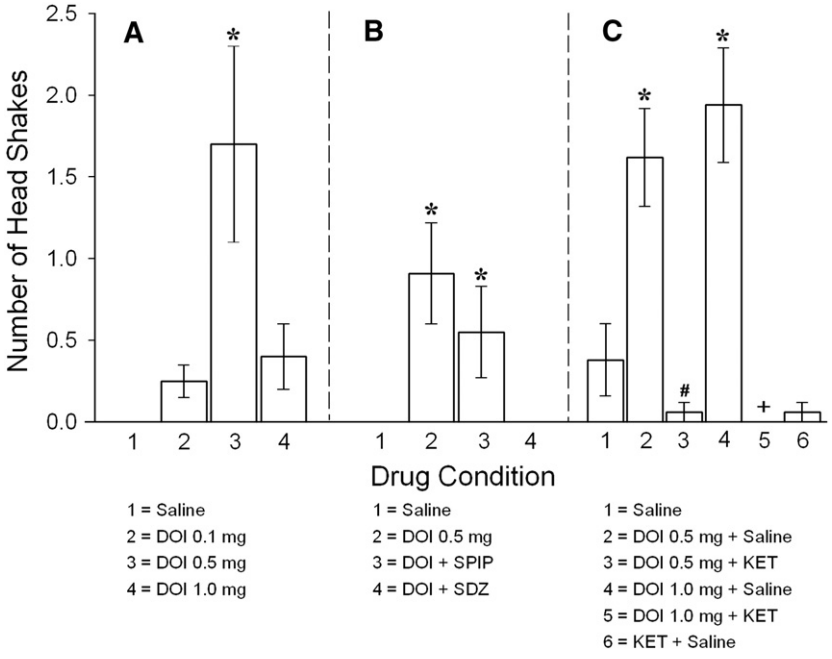

Fig. 3. Number of instances of head shaking in the open field following injections of saline control, DOI, spiperone (SPIP), SDZ SER-082 (SDZ), ketanserin (KET), or combinations of these. Panels A and B represent data from Experiment 1 and panel C contains data from Experiment 2. Designations of numbers on the *x*-axis representing drug condition are as in Fig. 1. Significance values: * = $p \leq 0.05$ relative to saline control, # = $p \leq 0.05$ relative to DOI 0.5 mg/kg + saline, + = $p \leq 0.05$ relative to DOI 1.0 mg/kg + saline.

5. Discussion

Tail pinch stress evoked an increase in the amount of time animals engaged in oral behavior directed at food. As seen previously in our laboratory (Hawkins et al., 2002) DOI reversed stress-evoked oral behavior in a dose-dependent fashion. This finding is consistent with the suggestion that 5-HT_{2A} and/or 5-HT_{2C} receptors are involved in the stress response and that activation of one or more of these receptors may reduce an animal's reactivity to stress. That selective blockade of the 5-HT_{2A} or 5-HT_{2C} receptor with spiperone or SDZ SER-082 respectively did not prevent the DOI effect suggests that DOI may act through both receptor subtypes and that joint antagonism of both subtypes may be necessary to prevent the action of DOI.

In the open field DOI increased the number of head shakes. This effect has been reported previously by us (Hawkins et al., 2002) and others (e.g., Koskinen et al., 2003; Mitchell et al., 2003) and is regarded as a pharmacological effect of DOI unrelated to stress. The convention is to attribute head shaking behavior to activation of the 5-HT_{2A} receptor (e.g., Dursun and Handley, 1996; Islam et al., 2004; Mitchell, et al., 2003). We observed, however, that the 5-HT_{2A} antagonist spiperone did not alter DOI-induced head shaking, while the 5-HT_{2C} antagonist SDZ SER-082 abolished it completely. These findings suggest that the 5-HT_{2C} receptor may play an important role in mediating head shaking behavior in rodents.

A reduction in rearing behavior in the open field was observed in one group of animals after injection of medium and high doses of DOI. While this finding could be interpreted as a decrease in escape behavior and, therefore, indicative of a reduction in stress reactivity (Dielenberg and McGregor 2001), reductions in rearing are traditionally considered to be an indication of increased stress responding (e.g., Cornwell-Jones, et al., 1992; Rogers et al., 2000; To and Bagdy 1999). Whether this effect of DOI is differentially attributable to 5-HT_{2A} or 5-HT_{2C} receptors could not be determined in the present experiment as the main effect for drug treatment only approached significance ($p=0.06$) on this variable in the group of animals given the medium dose of DOI in conjunction with selective antagonists.

Finally, while no dose of DOI alone had an effect on line crossing or freezing behavior, when given in conjunction with SDZ SER-082 the number of lines crossed was reduced and the duration of freezing behavior was increased. Whether these are related to the stress response or represent nonspecific pharmacological effects remains to be determined.

6. Experiment 2

Experiment 2 was performed to explore the possibility that the effects of systemically administered DOI on stress-evoked behavior could be blocked by simultaneous antagonism of both the 5-HT_{2A} and 5-HT_{2C} receptors. Two studies employing a between-subjects protocol were conducted. In Study 1, effects of the 5-$HT_{2A/2C}$ antagonist ketanserin (KET) were tested. Four groups of animals were used: saline control ($n=11$), 0.5 mg/kg KET ($n=11$), 2.5 mg/kg KET ($n=10$), and 5.0 mg/kg KET ($n=10$). Study 2 evaluated the possibility that effects of DOI on stress responding would be blocked by coadministration of KET. Six groups of animals were tested: saline control ($n=16$), 0.5 mg/kg DOI + saline ($n=16$), 1.0 mg/kg DOI + saline ($n=16$), 5.0 mg/kg KET + saline ($n=16$), 0.5 mg/kg DOI + 5.0 mg/kg KET ($n=16$), and 1.0 mg/kg DOI + 5.0 mg/kg KET ($n=16$). The 0.5 and 1.0 mg/kg doses of DOI are referred to as "low" and "high" doses respectively in the presentation of findings below.

6.1. Injection procedure

Animals in Study 1 received a single injection of KET or saline using the protocol previously described. In Study 2 a double injection procedure was used. Groups that were administered DOI + KET were injected with DOI first and KET (or saline) immediately afterward. Animals that received a single drug (KET or DOI) received an injection of saline immediately afterward to control for possible effects of repeated injection. The saline control group also received two injections. All injections were s.c. and injection volume was 1.0 ml/kg for each injection. After the second injection, animals were returned to their cages for 30 min prior to tail pinch testing.

7. Results

Study 1: MANOVA revealed that ketanserin had no effect on stress-evoked behavior [$F(36, 80)=1.285$, $p=0.176$]. Similarly, ANOVA and X^2 analysis demonstrated no effect of KET on rotarod performance or flat body posture ($p>0.05$ for both comparisons). Therefore, the highest dose of KET (5.0 mg/kg) was paired with DOI in Study 2.

Study 2: Drug administration significantly altered behavioral responding [MANOVA $F(65, 372)=3.82$, $p<0.001$]. In the tail pinch condition three behaviors were affected: oral behavior directed at food [$F(5, 90)=5.59$, $p<0.001$], eating [$F(5, 90)=3.44$, $p=0.007$], and vocalization [$F(5, 90)=4.51$, $p=0.001$]. A reduction in grooming approached significance [$F(5, 90)=2.21$, $p=0.06$]. In the open field, duration of rearing [$F(5, 90)=12.37$, $p<0.001$], number of head shakes [$F(5, 90)=16.98$. $p<0.001$], and occurrence of flat body posture [X^2 (5)=62.3, $p<0.001$] were significantly affected. No dose of DOI or KET, alone or in combination, resulted in significant motor impairment as measured by rotarod performance.

Oral behavior directed at food during tail pinch is depicted in Fig. 1, panel C. As in Experiment 1, both the 0.5 mg/kg and the 1.0 mg/kg doses of DOI significantly reduced responding relative to saline control ($p=0.021$ and $p=0.003$ respectively). The effects of these two doses did not differ from one another. While KET administered by itself did not alter responding ($p>0.05$ relative to saline), coadministration of KET with both the low and the high doses of DOI resulted in significantly greater amounts of oral behavior relative to their respective DOI + saline doses ($p=0.001$ and $p=0.043$ respectively), and a reversal of the effects of DOI ($p>0.05$ relative to saline for both doses).

The pattern of results seen in oral behavior directed at food was repeated in stress-evoked eating (Fig. 4). Eating was significantly reduced by the low ($p=0.007$) and high ($p=0.001$) doses of DOI. This effect was reversed by concomitant KET injection for the low dose of DOI ($p=0.329$ relative to saline), but not the high dose ($p=0.027$ relative to saline). Ketanserin itself had no effect on responding ($p>0.05$ relative to saline), and the amount of food eaten following

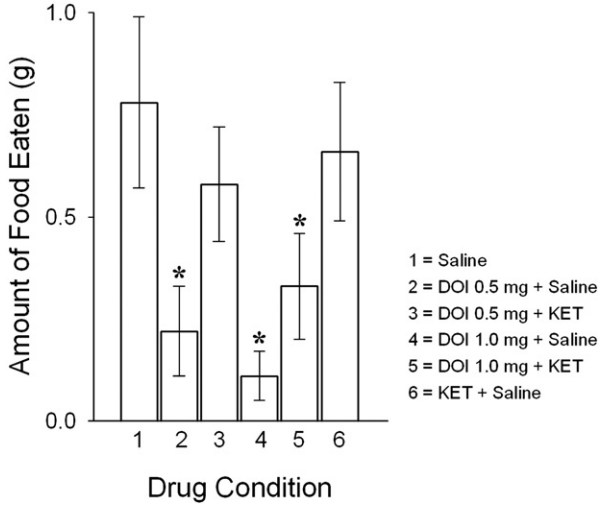

Fig. 4. Grams of food consumed during tail pinch stress following injections of saline control, DOI, ketanserin (KET), or combinations of these. * = $p \leq 0.05$ relative to saline control.

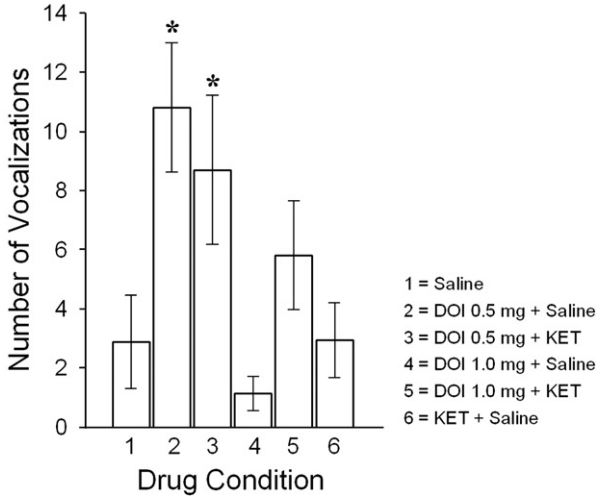

Fig. 5. Number of instances of vocalization during tail pinch stress following injections of saline control, DOI, ketanserin (KET), or combinations of these. Designations of numbers on the *x*-axis representing drug condition are as in Fig. 4. * = $p \leq 0.05$ relative to saline control.

either combined DOI + KET dose was statistically indistinguishable from KET alone.

Vocalization was increased by the low ($p=0.002$), but not the high dose, of DOI (Fig. 5). Ketanserin did not alter vocalization and did not significantly reverse the effect of the low dose of DOI ($p=0.023$ relative to saline).

Duration of rearing in the open field was significantly reduced by the low ($p=0.001$) and high ($p<0.001$) doses of DOI (Fig. 2, panel B). This pattern of results was also seen in Experiment 1. Unlike Experiment 1, however, concomitant injection of the 5-HT antagonist reversed the DOI effect. Coadministration of KET with DOI resulted in a significant increase in rearing for both the low and high doses of DOI relative to their respective DOI + saline doses ($p<0.001$ for both comparisons). Duration of rearing during combined DOI + KET injection was no different than that seen in the saline condition. Ketanserin by itself also had no effect on rearing.

While in Experiment 1 frequency of head shaking was increased by the 0.5 mg/kg dose of DOI only, both this and the 1.0 mg/kg dose evoked increased head shaking in Experiment 2 (see Fig. 3, panel C). This effect was significantly reduced by coadministration of KET ($p \leq 0.001$ for all comparisons). Ketanserin + saline had no effect on head shaking and frequency of head shaking returned to control levels when KET was given with DOI ($p>0.05$ for all comparisons).

A significant increase in the occurrence of flat body posture was observed following DOI injection [X^2 (5)=62.3, $p<0.001$] (data not shown). Flat body posture occurred in 50% of the animals given the low dose of DOI and in 100% of the animals given the high dose. When KET was coadministered with DOI the rate of occurrence dropped to 6% for both groups. Postural change was not observed in any of the animals given KET + saline.

8. General discussion

The pattern of effects produced by DOI during exposure to tail pinch stress suggests that activation of 5-HT_2 systems results in decreased reactivity. The only instance of behavior indicative of increased reactivity to tail pinch stress following DOI was an increase in vocalization. This was seen in Experiment 2 only and was not dose-related. Conversely, across three iterations (two in Experiment 1 and one in Experiment 2) DOI resulted in a consistent, dose-related reduction in oral behavior directed at food. In Experiment 2 a reduction in stress-induced eating was also seen and a reduction in grooming behavior approached significance. In addition to these variables we have previously observed reductions in tail pinch-induced gnawing, teeth chattering, and grooming following DOI (Hawkins et al., 2002). Similarly, others have reported that DOI results in decreased escape behavior (de Paula Soares and Zangrossi 2004; Nic Dhonnchadha et al., 2003a,b; Onaivi et al., 1995), increased exploratory behavior (Peng et al., 2004), reduced ultrasonic vocalization to foot shock (De Vry et al., 1993; Sanchez, 1993; Schreiber et al., 1998; Winslow and Insel, 1991), and anxiolytic-like effects in the four plates test (Nic Dhonnchadha et al., 2003a; Petit-Demouliere et al., 2008; Ripoll et al., 2006).

DOI is a phenethylamine (mescaline-like) hallucinogen (see Fantegrossi et al., 2008 for a review) which, like other hallucinogens, has been shown by some to produce anxiogenic responses (e.g. Bull et al., 2004). That DOI was shown to decrease reactivity to stress in the present study is consistent with the reports of others cited above and may be related to dose and species effects. Onaivi and colleagues (Onaivi et al., 1995), for example, showed that systemic administration of DOI in doses identical to those employed here (0.1, 0.5, and 1.0 mg/kg) produced dose-related anxiolytic responses in rats tested in the elevated plus maze. Additionally, these effects were reversed by ketanserin. Higher doses of DOI (2.5 and 5.0 mg/kg), however, resulted in anxiogenic reactions. The same pattern of anxiogenic reaction to high doses of DOI and anxiolytic effects at low doses (which was reversed by ketanserin) was observed in ICR mice. In DBA/2 mice, however, all doses of DOI produced an anxiogenic effect while in C57/BL6 mice DOI resulted in anxiolytic reactions at all doses.

The possibility that the DOI-induced reductions in responding observed in the current experiments were attributable to a non-specific motor impairment is militated against by the fact that administration of DOI by itself did not significantly increase the duration of freezing behavior, nor did it reduce gnawing, oral behavior not directed at food, rotarod performance or locomotion in the open field at any of the doses tested. While our observation that DOI alone did not affect locomotion confirms previous work (Raghavendra and Kulkarni, 2000; Redrobe and Bourin, 1997), increases (Darmani et al., 1996; Granoff and Ashby, 1998) and decreases (Kaur and Ahlenius, 2000; Krebs-Thomson and Geyer, 1996) in locomotion following administration of DOI have also been reported.

In the open field reductions in rearing were observed in both experiments following application of the 0.5 mg/kg and 1.0 mg/kg (but not 0.1 mg/kg) doses of DOI. As mentioned previously, whether this finding is to be interpreted as indicative of a decrease (Dielenberg and McGregor, 2001) or an increase (Cornwell-Jones et al., 1992; Rogers et al., 2000; To and Bagdy, 1999) in stress reactivity is a matter of some dispute. The conventional interpretation is that a reduction in rearing suggests increased stress responding.

In both experiments animals that received DOI consistently displayed a significant increase in the frequency of head shakes. This behavior is usually considered to be a pharmacological action unrelated to stress reactivity. However, exposure to stress has been reported to influence the number of head shakes elicited by DOI, with both increases (Brotto et al., 1998; Chaouloff et al., 1994; Takao et al., 1995) and decreases (Izumi et al., 2002; Pericic, 2003; Yamada et al., 1993; Yamada et al., 1995) being reported. It is also the case that head shakes are generally attributed to activation of the 5-HT_{2A} receptor (e.g., Dursun and Handley, 1996; Islam et al., 2004; Mitchell et al., 2003). We observed, however, that DOI-induced head shaking was abolished by joint antagonism of 5-$HT_{2A,2C}$ receptors with ketanserin or 5-HT_{2C} antagonism with SDZ SER-082, but the 5-HT_{2A} antagonist spiperone was ineffective. These findings suggest that the 5-HT_{2C} receptor, or combined 5-$HT_{2A,2C}$ receptor activity, may be important in this effect of DOI.

Though head shaking was the only behavior evoked by DOI that was reversed by selective antagonism of the 5-HT_{2A} or 5-HT_{2C} receptor, simultaneous antagonism of both receptor subtypes with ketanserin consistently reversed the behavioral effects of DOI. Significant blockade of the DOI effect was observed in measurements

of oral behavior directed at food and the amount of food eaten in the tail pinch condition, as well as rearing, head shakes, and flat body posture in the open field. The only DOI-induced behavior that was not reversed by ketanserin was vocalization. While this is the first report that ketanserin reverses the effects of DOI on behaviors evoked by tail pinch and open field stressors, it is consistent with other reports of ketanserin blockade of the actions of DOI or other 5-HT_2 agonists. In this regard, ketanserin has been shown to block 5-HT_2-induced anxiolytic effects on ultrasonic vocalizations (Schreiber et al., 1998), social interactions (Nakamura and Kurasawa, 2001; Tadano et al., 2001), and behaviors in the elevated T-maze (de Paula Soares and Zangrossi, 2004) and the elevated plus maze (Onaivi et al., 1995).

Ketanserin binds with high affinity to both α_1-adrenoceptors and histamine H_1 receptors (see Table 1) and is believed to be antagonistic at both (Ghoneim et al., 2006; Sathi et al., 2008). Given that H_1 and α_1-adrenoceptor antagonists have been shown to produce anxiolytic responses (see Millan, 2003 for review) it seems unlikely that the ability of ketanserin to reverse the actions of DOI in the results reported here is attributable to these receptor subtypes. These results indicate that the behavioral consequences of DOI, and other 5-HT_2 agonists, in these paradigms may be largely attributable to combined activation of both 5-HT_{2A} and 5-HT_{2C} receptors.

References

Antelman SM, Szechtman H. Tail pinch induces eating in sated rats which appears to depend on nigrostriatal dopamine. Science 1975;189:731–3.

Blessing WW, Seaman B. 5-Hydroxytryptamine $_{2A}$ receptors regulate sympathetic nerves constricting the cutaneous vascular bed in rabbits and rats. Neuroscience 2003;117:939–48.

Boess FG, Martin IL. Molecular biology of 5-HT receptors. Neuropharmacology 1994;33 (3–4):275–317.

Bogeso KP, Arnt J, Boeck V, Christensen AV, Hyttel J, Jensen KG. Antihypertensive activity in a series of 1-piperazino-3-phenylindans with potent 5-HT2-antagonistic activity. J Med Chem 1988;31(12):2247–56.

Broderick PA, Olabisi OA, Rahni DN, Zhou Y. Cocaine acts on accumbens monoamines and locomotor behavior via a 5-$HT_{2A/2C}$ receptor mechanism as shown by ketanserin: 24-h follow-up studies. Prog Neuro-Psychopharmacol Biol Psychiatry 2004;28:547–57.

Brotto LA, Gorzalka BB, Hanson LA. Effects of housing conditions and 5-HT2A activation on male rat sexual behavior. Physiol Behav 1998;63:475–9.

Bull EJ, Hutson PH, Fone KCF. Decreased social behaviour following 3,4-methylenedioxymethamphetamine (MDMA) is accompanied by changes in 5-HT_{2A} receptor responsivity. Neuropharmacology 2004;46:202–10.

Chaouloff F, Baudrie V, Coupry I. Effects of chlorisondamine and restraint on cortical [3H]ketanserin binding, 5-HT2A receptor-mediated head shakes, and behaviours in models of anxiety. Neuropharmacology 1994;33:449–56.

Cornwell-Jones CA, Palfai T, Krasenbaum D, Byer Jr E, Clark R, Kinnard K. Housing influences exploration and social interaction of control and DSP-4-treated rats. Physiol Behav 1992;52(2):271–6.

Czech DA, Klosterman AE, Le Sueur KT. N^G-nitro-L-arginine methyl ester reduces stress-related feeding in the rat tail-pinch model. Pharmacol Biochem Behav 1998;60 (1):91–6.

Darmani NA, Shaddy J, Gerdes CF. Differential ontogenesis of three DOI-induced behaviors in mice. Physiol Behav 1996;60:1495–500.

Dave KD, Harvey JA, Aloyo VJ. A novel behavioral model that discriminates between 5-HT_{2A} and 5-HT_{2C} receptor activation. Pharmacol Biochem Behav 2002;72(1–2):371–8.

de Paula Soares V, Zangrossi Jr H. Involvement of 5-HT1A and 5-HT2 receptors of the dorsal periaqueductal gray in the regulation of the defensive behaviors generated by the elevated T-maze. Brain Res Bull 2004;64(2):181–8.

De Vry J, Benz U, Schreiber R, Traber J. Shock-induced ultrasonic vocalization in young adult rats: a model for testing putative anti-anxiety drugs. Eur J Pharmacol 1993;249:331–9.

Dielenberg RA, McGregor IS. Defensive behavior in rats towards predatory odors: a review. Neurosci Biobehav Rev 2001;25(7–8):597–609.

Dursun SM, Handley SL. Similarities in the pharmacology of spontaneous and DOI-induced head-shakes suggest 5HT2A receptors are active under physiological conditions. Psychopharmacology (Berl) 1996;128(2):198–205.

Duxon MS, Kennett GA, Lightowler S, Blackburn TP, Fone KC. Activation of 5-HT2B receptors in the medial amygdala causes anxiolysis in the social interaction test in the rat. Neuropharmacology 1997;36(4–5):601–8.

Fantegrossi WE, Murnane KS, Reissig CJ. The behavioral pharmacology of hallucinogens. Biochem Pharmacol 2008;75:17–33.

Filip M, Bubar MJ, Cunnigham KA. Contribution of serotonin (5-hydroxytryptamine; 5-HT) 5-HT_2 receptor subtypes to the hyperlocomotor effects of cocaine: Acute and chronic pharmacological analyses. J Pharmacol Exp Ther 2004;310(3):1246–54.

Fray PJ, Koob GF, Iversen SD. Tail-pinch-elicited behavior in rats: preference, plasticity, and learning. Behav Neural Biol 1982;36:108–25.

Ghoneim OM, Legerea JA, Golbraikha A, Tropsha A, Booth RG. Novel ligands for the human histamine H1 receptor: synthesis, pharmacology, and comparative molecular field analysis studies of 2-dimethylamino-5-(6)-phenyl-1,2,3,4-tetrahydronaphthalenes. Bio Med Chem 2006;14:6640–58.

Giorgi O, Lecca D, Piras G, Driscoll P, Corda MG. Dissociation between mesocortical dopamine release and fear-related behaviours in two psychogenetically selected lines of rats that differ in coping strategies to aversive conditions. Eur J Neurosci 2003;17:2716–26.

Granoff MI, Ashby Jr CR. The effect of the repeated administration of the compound 3,4-methylenedioxymethamphetamine on the response of rats to the 5-HT2A,C receptor agonist (+/−)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). Neuropsychobiology 1998;37:36–40.

Glusa E, Pertz HH. Further evidence that 5-HT induced relaxation of pig pulmonary artery is mediated by endothelial 5-HT(2B) receptors. Br J Pharmacol 2000;130 (3):692–8.

Griebel G, Perrault G, Sanger DJ. A comparative study of the effects of selective and non-selective 5-HT2 receptor subtype antagonists in rat and mouse models of anxiety. Neuropharmacology 1997;36(6):793–802.

Hawkins MF, Uzelac SU, Baumeister AA, Hearn JK, Broussard JI, Guillot TS. Behavioral responses to stress following central and peripheral injection of the 5-HT(2) agonist DOI. Pharmacol Biochem Behav 2002;73(3):537–44.

Hoyer D, Clarke DE, Fozrd JR, Hartig PR, Martin GR, Mylecharane EJ, et al. VII. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol Rev 1994;46(2):157–203.

Islam A, Thompson KS, Akhtar S, Handley SL. Increased 5-HT2A receptor expression and function following central glucocorticoid receptor knockdown in vivo. Eur J Pharmacol 2004;502(3):213–20.

Izumi T, Suzuki K, Inoue T, Li XB, Maki Y, Muraki I, et al. Long-lasting change in 5-HT2A receptor-mediated behavior in rats after a single foot shock. Eur J Pharmacol 2002;452:199–204.

Kaur P, Ahlenius S. Non-serotonergic potentiation by (−)-pindolol of DOI-induced forward locomotion in rats: possible involvement of beta-adrenoceptors? J Neural Transm 2000;107:903–17.

Kennett GA, Wood MD, Bright F, Trail B, Riley G, Holland V, et al. SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist. Neuropharmacology 1997;36(4–5):609–20.

Knight AR, Misra A, Quirk K, Benwell K, Revell D, Kennett G, et al. Pharmacological cauterization of the agonist radioligand binding site of 5-HT(2A), 5-HT(2B) and 5-HT(2C) receptors. Naunyn Schmiedbergs Arch Pharmacol 2004;370(2):114–23.

Koskinen T, Haapalinna A, Sirvio J. Alpha-adrenoceptor-mediated modulation of 5-HT2 receptor agonist induced impulsive responding in a 5-choice serial reaction time task. Pharmacol Toxicol 2003;92(5):214–25.

Krebs-Thomson K, Geyer MA. The role of 5-HT(1A) receptors in the locomotor-suppressant effects of LSD: WAY-100635 studies of 8-OH-DPAT, DOI and LSD in rats. Behav Pharmacol 1996;7:551–9.

Kursar JD, Nelson DL, Wainscott DB, Baez M. Molecular cloning, functional expression, and mRNA tissue distribution of the human 5-hydroxytryptamine2B receptor. Mol Pharmacol 1994;46(2):227–34.

Lê AD, Tomkins D, Higgins G, Quan B, Sellers EM. Effects of 5-HT_3, D_1 and D_2 receptor antagonists on ethanol- and cocaine-induced locomotion. Pharmacol Biochem Behav 1997;57(1–2):325–32.

Massé F, Nic Dhonnchadha BA, Hascoët M, Bourin M. Anxiolytic-like effect of 5-HT_2 ligands and benzodiazepines co-administration: comparison of two animal models of anxiety (the four-plate test and the elevated plus maze). Behav Brain Res 2007;177:214–26.

Millan MJ. The neurobiology and control of anxious states. Prog Neurobiol 2003;70:83–244.

Millan MJ, Canton H, Gobert A, Lejeune F, Rivet JM, Bervoets K, et al. Novel benzodiazepines acting as antagonists at 5-HT1A autoreceptors: a comparative pharmacological characterization with proposed 5-HT1A antagonists. J Pharmacol Exp Ther 1994;268(1):337–52.

Mitchell PJ, Fairhall SJ, Fretcher A, Redfern PH. Effects of single and repeated electroconvulsive shock on the social and agonistic behaviour of resident rats. Neuropharmacology 2003;44(7):911–25.

Miyata S, Hirano S, Kamei J. Diabetes inhibits the DOI-induced head twitch response in mice. Psychopharmacology 2004;177:224–9.

Mora PO, Netto CF, Graeff FG. Role of 5-HT2A and 5-HT2C receptor subtypes in the two types of fear generated by the elevated T-maze. Pharmacol Biochem Behav 1997;58(4):1051–7.

Nakamura K, Kurasawa M. Anxiolytic effects of aniracetam in three different mouse models of anxiety and the underlying mechanism. Eur J Pharmacol 2001;420:33–43.

Nic Dhonnchadha BA, Bourin M, Hascoet M. Anxiolytic-like effects of 5-HT2 ligands on three mouse models of anxiety. Behav Brain Res 2003a;140:203–14.

Nic Dhonnchadha BA, Hascoet M, Jolliet P, Bourin M. Evidence for a 5-HT2A receptor mode of action in the anxiolytic-like properties of DOI in mice. Behav Brain Res 2003b;147:175–84.

Njunge K, Handley SL. Effects of 5-HT uptake inhibitors, agonists and antagonists on the burying of harmless objects by mice; a putative test of anxiolytic agents. Br J Pharmacol 1991;104(1):105–12.

Onaivi ES, Bishop-Robinson C, Darmani NA, Sanders-Bush E. Behavioral effects of (+/−)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, (DOI) in the elevated plus-maze test. Life Sci 1995;57(26):2455–66.

Peng WH, Wu CR, Chen CS, Chen CF, Leu ZC, Hsieh MT. Anxiolytic effect of berberine on exploratory activity of the mouse in two experimental anxiety models: interaction with drugs acting at 5-HT receptors. Life Sci 2004;75:2451–62.

Pericic D. Swim stress inhibits 5-HT2A receptor-mediated head twitch behaviour in mice. Psychopharmacology (Berl) 2003;167:373–9.

Petit-Demouliere B, Hascoet M, Bourin M. Factors triggering abolishment of benzodiazepines effects in the four-plate test–retest in mice. Eur Neuropsychopharm 2008;18:41–7.

Rademacher DJ, Anderson AP, Steinpreis RE. Acute effects of amperozide and paroxetine on social cohesion in male conspecifics. Brain Res Bull 2002;58(2):187–91.
Raghavendra V, Kulkarni SK. Melatonin reversal of DOI-induced hypophagia in rats; possible mechanism by suppressing 5-HT(2A) receptor-mediated activation of HPA axis. Brain Res 2000;860:112–8.
Redrobe JP, Bourin M. Effects of pretreatment with clonidine, lithium and quinine on the activities of antidepressant drugs in the mouse tail suspension test. Fundam Clin Pharmacol 1997;11:381–6.
Ripoll N, Hascoet M, Bourin M. Implication of 5-HT_{2A} subtype receptors in DOI activity in the four-plates test–retest paradigm in mice. Behav Brain Res 2006;166:131–9.
Rogers DC, Costall B, Domeney AM, Gerrard PA, Greener M, Kelly ME, Hagan JJ, Hunter AJ. Anxiolytic profile of ropinirole in the rat, mouse and common marmoset. Psychopharmacology (Berl) 2000;151(1):91–7.
Roth BL, Tandra S, Burgess LH, Sibley DR, Meltzer HY. D4 dopamine receptor binding affinity does not distinguish between typical and atypical antipsychotic drugs. Psychopharmacology (Berl) 1995;120(3):365–8.
Samarghandian S, Ohata H, Yamauchi N, Shibasaki T. Corticotropin-releasing factor as well as opioid and dopamine are involved in tail-pinch-induced food intake of rats. Neuroscience 2003;116(2):519–24.
Sanchez C. Effect of serotonergic drugs on footshock-induced ultrasonic vocalization in adult male rats. Behav Pharmacol 1993;4:269–77.
Sathi ZS, Anisuzzaman ASM, Morishima S, Suzuki F, Tanaka T, Yoshiki H, et al. Different affinities of native α_{1B}-adrenoceptors for ketanserin between intact tissue segments and membrane preparations. Eur J Pharmacol 2008;584:222–8.
Schreiber R, Melon C, De Vry J. The role of 5-HT receptor subtypes in the anxiolytic effects of selective serotonin reuptake inhibitors in the rat ultrasonic vocalization test. Psychopharmacology 1998;135(4):383–91.
Schwinn DA, Johnston GI, Page SO, Mosley MJ, Wilson KH, Worman NP, et al. Cloning and pharmacological characterization of human alpha-1-adrenergic receptors: sequence corrections and direct comparison with other species homologues. J Pharmacol Exp Ther 1995;272(1):134–42.
Sokal DH, Giarola AS, Large CH. Effects of GABA(B), 5-HT(1A), and 5-HT(2) receptor stimulation on activation and inhibition of the rat lateral amygdala following medial geniculate nucleus stimulation in vivo. Brain Res 2005;1031(1):141–50.
Swerdlow NR, Keith VA, Braff DL, Geyer MA. Effects of spiperone, raclopride, SCH 23390 and clozapine on apomorphine inhibition of sensorimotor gating of the startle response in the rat. J Pharacol Exp Ther 1991;256(2):530–6.
Tadano T, Hozumi M, Satoh N, Oka R, Hishinuma T, Mizugaki M, et al. Central serotonergic mechanisms on head twitch response induced by benzodiazepine receptor agonists. Pharmacology 2001;62:157–62.
Takao K, Nagatani T, Kitamura Y, Kawasaki K, Hayakawa H, Yamawaki S. Chronic forced swim stress of rats increases frontal cortical 5-HT2 receptors and the wet-dog shakes they mediate, but not frontal cortical beta-adrenoceptors. Eur J Pharmacol 1995;294:721–6.
Tang L, Todd RD, Heller A, O'Malley KL. Pharmacological and functional chacterization of D2, D3 and D4 dopamine receptors in fibroblast and dopaminergic cell lines. J Pharmacol Exp Ther 1994;268(1):495–502.
To CT, Bagdy G. Anxiogenic effect of central CCK administration is attenuated by chronic fluoxetine or ipsapirone treatment. Neuropharmacology 1999;38(2):279–82.
Toll L, Berzetei-Gurske IP, Polgar WE, Brandt SR, Adapa ID, Rodriguez L, et al. Standard binding and functional assays related to medications development division testing for potential cocaine and opiate narcotic treatment medications. NIDA Res Monogr 1998;178:440–66.
Wax J, McLean JR, Tessman DK, McCarthy DA, Nuite JA. Duration of antinociceptive action of morphine, methadone and α–*l*–acetylmethadol subcutaneously in rats. Res Com Chem Path Pharmacol 1975;12(4):613–20.
Winslow JT, Insel TR. Serotonergic modulation of the rat pup ultrasonic isolation call: Studies with 5HT1 and 5HT2 subtype-selective agonists and antagonists. Psychopharmacology (Berl) 1991;105:513–20.
Yamada S, Nankai M, Toru M. Acute immobilization stress reduces (+/−) DOI induced 5-HT2-mediated head shakes in rats. Jpn J Psychiatry Neurol 1993;47:414–5.
Yamada S, Watanabe A, Nankai M, Toru M. Acute immobilization stress reduces (+/−) DOI-induced 5-HT2A receptor-mediated head shakes in rats. Psychopharmacology (Berl) 1995;119:9–14.